

Diagnostic Molecular Microbiology

PRINCIPLES AND APPLICATIONS

Edited by

David H. Persing, M.D., Ph.D.

Section of Clinical Microbiology, Mayo Clinic, Rochester, Minnesota

Thomas F. Smith, Ph.D.

Section of Clinical Microbiology, Mayo Clinic, Rochester, Minnesota

Fred C. Tenover, Ph.D.

National Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia

Thomas J. White, Ph.D.

Roche Molecular Systems, Alameda, California

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Sample Preparation Methods



LARRY GREENFIELD AND THOMAS J. WHITE

Although there has been progress in simplifying the release and purification of bacterial or viral nucleic acids from clinical specimens, many research procedures are still unsuitable for the clinical laboratory and a universal automated method for use with any specimen has not yet been devised. In this chapter, we review some of the basic principles that have been learned to date which may guide and encourage the reader to develop further improvements that eliminate the requirements for hazardous solutions, centrifugations, and multiple steps. A variety of approaches which may be appropriate for certain specimens and pathogens but not for others are then described. Finally, each specimen type (e.g., whole blood, urine, sputum) is discussed with regard to specific protocols and pathogens.

Basic Principles

The ideal sample preparation method represents a trade-off between the requirements for the optimal method, the clinical specimen, and the target (Table 1). Although many of these considerations are interrelated, selection of a few crucial items helps define many others. Once the target organism is selected, the clinical pathogenesis of the infection generally dictates the appropriate specimen and number of microorganisms likely to be present. Determination of the desired assay sensitivity and the number of tests to be performed on the processed sample then dictates the required volume of specimen to be processed.

Sample Size Versus Target Copy Number

Microbiological culture as a "gold standard" has directed our selection of the appropriate specimen for many infectious diseases, e.g., blood or plasma for human immunodeficiency virus (HIV) and hepatitis C virus (HCV) and endocervical swabs for chlamydiae. For other pathogens, e.g., *Borrelia burgdorferi*, the optimal specimen for diagnosing each stage of infection has not yet been identified. For molecular diagnostic tests that are based on amplification, a single copy or molecule of the genetic target from the pathogen, if present in the reaction, can be detected in a fully optimized procedure (51, 70). To maximize the chance of diagnosing an infection, the largest convenient sample volume should be screened. However, since typical molecular diagnostic test reaction volumes are 100 μ l or less, one is faced with a choice between complex target concentration steps (e.g., ethanol precipitation, nucleic acid target capture, and centrifugation) and lowered assay sensitivity. Hence, if no amplifiable target is detected

Table 4. Fundamental goals of sample preparation protocols

Release of nucleic acid from bacteria, viruses, or fungi
Stabilization of nucleic acid against degradation
Removal of amplification inhibitors
Concentration of target into a small volume
Placement of target into an aqueous environment compatible with amplification

once an active infection has cleared. A study of the course of bacteremia by culture of specimens following oral trauma showed that viable bacteria were rapidly cleared from the blood (40). In one study of chlamydia infections following treatment, results of PCR and culture showed perfect concordance (23). However, these may be best-case scenarios; other studies have documented late persistence of nucleic acid (64). In some cases it is beneficial to detect dead microorganisms, e.g., when an inaccessible reservoir of live organisms sheds bacteria that are rapidly cleared. Many more longitudinal studies of treatment to cure for various diseases will be necessary to determine the clinical significance of DNAemia.

Overview of Approaches

Sample preparation methods can be divided into a number of generic steps (Table 4). The requirement for each step will depend on the organism and specimen. The release of nucleic acid may be easy for viruses and some bacteria (e.g., *Mycoplasma* species) but difficult for other bacteria (e.g., *Mycobacterium tuberculosis*) and fungi. RNA is more difficult to stabilize than DNA. More steps may be required to remove inhibitors from some specimens (e.g., sputum and blood) than from others (e.g., urine and CSF). Some specimens (e.g., sputum for *M. tuberculosis* and *Legionella pneumophila* and blood for sepsis) may require a greater degree of concentration than others (e.g., urethral swabs and urine for *Chlamydia* or *Gonococcus* species) to achieve the required sensitivity.

There are a variety of methods for the release of nucleic acid from microorganisms, including boiling in distilled water or PCR buffer (76), detergents with or without heat (76), sodium hydroxide with heat (13), freeze-thaw (15), SDS-proteinase K (51), perchloric acid (76), enzymes (30), sonication (15), and heat (55). Enzymatic digestion may be less desirable in that there may be components in the sample which prevent the action of the enzyme. For example, lysozyme has been used on liquified sputum (30), but sputum has a high content of mucopolysaccharides. Lysozyme is unstable following reduction (77) and forms complexes with dextrans and proteins (62). Many of the current and anticipated protocols require some method of separation for concentration of nucleic acid or removal of amplification inhibitors. Potential methods for separation include centrifugation, separation by magnetic particles, and separation by filtration.

Crude Lysis

The simplest sample preparation method would entail only a crude lysis. However, such a method would require a high concentration of target in the specimen and/or small amounts of amplification inhibitors. If no other separation step is included, the volume of the specimen to be processed is limited by the volume of the amplification reaction. Simple lysis methods typically use detergents such as SDS or Triton X-100, chaotropes such as guanidinium isothiocya-

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nate or sodium iodide, proteases such as proteinase K (which must be inactivated before the sample is added to the diagnostic reaction mixture) (42), substances such as saponin which lyse erythrocytes and leukocytes (e.g., the Wampole Isostat Microbial System), or heat (33). Such methods are generally suitable when the clinically significant number of infectious organisms per sample volume is large (e.g., *Chlamydia trachomatis* in endocervical swabs), so that the lysed specimen does not require significant further dilution (57). If the level of target is low, it is frequently necessary to remove amplification inhibitors by additional extraction (phenol-chloroform) steps or concentration of the target by alcohol precipitation. In addition, detergents are known to inhibit many enzymes, and high temperatures may result in degradation of nucleic acids (29).

Target Capture

Target capture or cycling offers the possible advantages of automation, universality for all specimens, and concentration of target into a small volume. This approach has been investigated by Gillespie et al. (36), Hunsaker et al. (44), and Lanciotti et al. (54). However, to date there are no published studies that demonstrate efficient capture and detection of fewer than 100 target molecules, and automated instruments and reagents for this approach are not yet commercially available. Derivatized magnetic particles can be coupled to oligonucleotide capture probes and combined with manual washing steps to remove extraneous materials (2, 19). These approaches have their own problems, though, since manual washing causes aerosols that may result in sample-to-sample contamination.

Other matrices have been tested for general adsorption of nucleic acids. Glass matrices, Sephadex, and diatomaceous earth bind nucleic acids in chaotropic solutions (11, 16, 59, 79). Following binding of the nucleic acids to the solid-phase matrix, the impurities and amplification inhibitors are removed by centrifugation and washing and the nucleic acids are eluted in an amplification-compatible buffer. Such approaches are promising since they are relatively simple, can be automated, and do not require hazardous reagents.

Finally, filtration may become a useful approach for certain kinds of specimens if it can be automated and made rapid (7a). Cost will be a problem unless disposable devices can be manufactured cheaply, and the requirement for a vacuum or centrifuge could be a burden for many laboratories.

Recommended Protocols for Various Specimens

Whole Blood

Even after it is decided that the desired specimen for a given target is blood, there still remain a number of choices: plasma, serum, whole blood, leukocyte fractions, etc. Furthermore, there is a choice of anticoagulants if the specimen is plasma: EDTA, heparin, or citrate. The anticoagulant used for plasma collection and the method of storage may affect the ability of the assay to detect the presence of target sequences (17, 80). Heparin was found to inhibit the activity of both murine leukemia virus reverse transcriptase and *Taq* DNA polymerase (46). In addition, the inhibitory effect of heparin does not appear to be removed by extraction of RNA by a modification of the acid-phenol-guanidinium method. For EDTA-containing tubes, it is recommended that the final concentration of EDTA be 1 to 2 mg/ml of blood (final concentration, 6.8 mM). For heparin-containing

References

1. Albert, J., and E. M. Fenyo. 1990. Simple, sensitive, and specific detection of human immunodeficiency virus type 1 in clinical specimens by polymerase chain reaction with nested primers. *J. Clin. Microbiol.* 28:1560-1564.
2. Albert, J., J. Wahlberg, J. Lundeberg, S. Cox, E. Sandstrom, B. Wabren, and M. Uhlen. 1992. Persistence of azidothymidine-resistant human immunodeficiency virus type 1 RNA genotypes in posttreatment sera. *J. Virol.* 66:5627-5630.
3. Anderson, B. E., J. W. Sumner, J. E. Dawson, T. Tzianabos, C. R. Greene, J. G. Olson, D. B. Fishbein, M. Olsen-Rasmussen, B. P. Holloway, E. H. George, and A. F. Azad. 1992. Detection of the etiologic agent of human ehrlichiosis by polymerase chain reaction. *J. Clin. Microbiol.* 30:775-780.
4. Baglaski, U., A. Ferrie, R. Watson, and D. Mack. 1990. Detection of hepatitis B virus, p. 348-355. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), *PCR Protocols: A Guide to Methods and Applications*. Academic Press, Inc., San Diego, Calif.
5. Balnaves, M. E., S. Nasloulas, H.-H. M. Dahl, and S. Forrest. 1991. Direct PCR from CVS and blood lysates for detection of cystic fibrosis and Duchenne muscular dystrophy deletions. *Nucleic Acids Res.* 19:1155.
6. Bauer, H. M., C. E. Greer, and M. M. Manos. 1992. Determination of genital human papillomavirus infection by consensus polymerase chain reaction amplification, p. 131-152. In C. S. Herrington and J. O. McGee (ed.), *Diagnostic Molecular Pathology: A Practical Approach*, vol. II. Oxford University Press, New York.
7. Bauwens, J. E., A. M. Clark, M. J. Loeffelholz, S. A. Herman, and W. E. Stamm. Diagnosis of *Chlamydia trachomatis* (CT) urethritis in men by PCR assay of first-void urine. Submitted for publication.
- 7a. Bej, A. K., M. H. Mahbubani, J. L. Dicesare, and R. M. Atlas. 1991. Polymerase chain reaction-gene probe detection of microorganisms by using filter-concentrated samples. *Appl. Environ. Microbiol.* 57:3529-3534.
8. Bloch, W. 1991. A biochemical perspective of the polymerase chain reaction. *Biochemistry* 30:2735-2747.
9. Boland, G. J., R. A. de Weger, M. G. J. Tilanus, C. Ververs, K. Bosboom-Kalsbcek, and G. C. de Gast. 1992. Detection of cytomegalovirus (CMV) in granulocytes by polymerase chain reaction compared with the CMV antigen test. *J. Clin. Microbiol.* 30:1763-1767.
10. Boom, R., C. J. A. Sol, R. Heijlink, P. M. E. Wertheim-van Dillen, and J. van der Noordaa. 1991. Rapid purification of hepatitis B virus DNA from serum. *J. Clin. Microbiol.* 29:1804-1811.
11. Boom, R., C. J. A. Sol, M. M. M. Sallmans, C. L. Jansen, and P. M. E. Wertheim-van Dillen. 1990. Rapid and simple method for purification of nucleic acids. *J. Clin. Microbiol.* 28:495-503.
12. Brian, M. J., M. Frosolono, B. E. Murray, A. Miranda, E. L. Lopez, H. F. Gomez, and T. G. Cleary. 1992. Polymerase chain reaction for diagnosis of enterohemorrhagic *Escherichia coli* infection and hemolytic-uremic syndrome. *J. Clin. Microbiol.* 30:1801-1806.
13. Brisson-Nöel, A., C. Aznar, C. Chureau, S. Nguyen, C. Pierre, M. Bartoli, R. Bonete, G. Pealoux, B. Gicquel, and G. Garrigue. 1991. Diagnosis of tuberculosis by DNA amplification in clinical practice evaluation. *Lancet* 338:364-366.
14. Brisson-Nöel, A., D. Lecossier, X. Nassif, B. Gicquel, V. Lévy-Frébault, and A. J. Hance. 1989. Rapid diagnosis of tuberculosis by amplification of mycobacterial DNA in clinical samples. *Lancet* i:1069-1071.
15. Buck, G. E., L. C. O'Hara, and J. T. Summersgill. 1992. Rapid, simple method for treating clinical specimens containing *Mycobacterium tuberculosis* to remove DNA for polymerase chain reaction. *J. Clin. Microbiol.* 30:1331-1334.
16. Buffone, G. J., G. J. Demmler, C. M. Schimbor, and J. Greer. 1991. Improved

- amplification of cytomegalovirus DNA from urine after purification of DNA with glass beads. *Clin. Chem.* 37:1945-1949.
17. Busch, M. P., J. C. Wilber, P. Johnson, L. Tobler, and C. S. Evans. 1992. Impact of specimen handling and storage on detection of hepatitis C virus RNA. *Transfusion* 32:420-425.
 18. Butcher, A., and J. Spadaro. 1992. Using PCR for detection of HIV-1 infection. *Clin. Immunol. Newsl.* 12:73-76.
 19. Chioldi, F., B. Keys, J. Albert, L. Hagberg, J. Lundeborg, M. Uhlen, E. M. Fenyo, and G. Norkrans. 1992. Human immunodeficiency virus type 1 is present in the cerebrospinal fluid of a majority of infected individuals. *J. Clin. Microbiol.* 30:1768-1771.
 20. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18:5294-5299.
 21. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156-159.
 22. Clulla, T. A., R. M. Sklar, and S. L. Hauser. 1988. A simple method for DNA purification from peripheral blood. *Anal. Biochem.* 174:485-488.
 23. Claas, H. C. J., J. H. T. Wagenvoort, H. G. M. Niesters, T. T. Tio, J. H. Van Rijsoort-Vos, and W. G. V. Quak. 1991. Diagnostic value of the polymerase chain reaction for *Chlamydia* detection as determined in a follow-up study. *J. Clin. Microbiol.* 29:42-45.
 24. Conway, B., L. J. Bechtel, K. A. Adler, R. T. D'Aquila, J. C. Kaplan, and M. S. Hirsch. 1992. Comparison of spot-blot and microtiter plate methods for the detection of HIV-1 PCR products. *Mol. Cell. Probes* 6:245-249.
 25. Cousins, D. V., S. D. Wilton, B. R. Francis, and B. L. Gow. 1992. Use of polymerase chain reaction for rapid diagnosis of tuberculosis. *J. Clin. Microbiol.* 30:255-258.
 - 25a. Cuypers, H. T. M., D. Bresters, I. N. Winkel, H. W. Reesink, A. J. Welaer, M. Houghton, C. L. van der Poel, and P. N. Lelle. 1992. Storage conditions of blood samples and primer selection affect the yield of cDNA polymerase chain reaction products of hepatitis C virus. *J. Clin. Microbiol.* 30:3220-3224.
 26. Dilworth, D. D., and J. R. McCarrey. 1992. Single-step elimination of contaminating DNA prior to reverse transcriptase-PCR. *PCR Methods Applic.* 1:279-282.
 27. Do, N., and R. P. Adams. 1991. A simple technique for removing plant polysaccharide contaminants from DNA. *BioTechniques* 10:162-166.
 28. Donofrio, J. C., J. D. Coenrod, J. N. Davidson, and R. F. Betts. 1992. Detection of influenza A and B in respiratory secretions with the polymerase chain reaction. *PCR Methods Applic.* 1:263-268.
 29. Eigner, J., H. Boedtker, and G. Michaels. 1961. The thermal degradation of nucleic acids. *Biochim. Biophys. Acta* 51:163-168.
 30. Eisenach, K. D., M. D. Sifford, M. D. Cave, J. H. Bates, and J. T. Crawford. 1991. Detection of *Mycobacterium tuberculosis* in sputum samples using a polymerase chain reaction. *Am. Rev. Respir. Dis.* 144:1160-1163.
 31. Faloona, F., S. Weiss, F. Ferre, and K. Mullis. 1990. Direct detection of HIV sequences in blood: high-gain polymerase chain reaction. *Abstr. 6th Int. Conf. AIDS.* 2:318.
 32. Frankel, G., L. Riley, J. A. Giron, J. Valmassol, A. Freidmann, N. Stokbine, S. Falkow, and G. K. Schoolnik. 1990. Detection of *Shigella* in feces using DNA amplification. *J. Infect. Dis.* 161:1252-1256.
 33. Frickhofen, N., and N. S. Young. 1991. A rapid method of sample preparation for detection of DNA viruses in human serum by polymerase chain reaction. *J. Virol. Methods* 35:65-72.
 34. Furukawa, K., and V. P. Bhavanandan. 1983. Influences of anionic polysaccharides on DNA synthesis in isolated nuclei and by DNA polymerase α : correlation of observed effects with properties of the polysaccharides. *Biochim. Biophys. Acta* 740:466-475.
 35. Gerritsen, M. J., T. Olyhoek, M. A. Smits, and B. A. Bokhout. 1991. Sample preparation method for polymerase chain reaction-based semiquantitative detection of

16050-0808280

- Leptospira interrogans* serovar Hardjo subtype hardjobovis in bovine urine. *J. Clin. Microbiol.* 29:2805-2808.
36. Gillespie, D., J. Thompson, and R. Solomon. 1989. Probes for quantitating subpicogram amounts of HIV-1 RNA by molecular hybridization. *Mol. Cell Probes* 3:73-86.
 37. Greer, C. E., S. L. Peterson, N. B. Kiviat, and M. M. Manos. 1991. PCR amplification from paraffin-embedded tissues: effects of fixative and fixation time. *Am. J. Clin. Pathol.* 95:117-124.
 38. Gustincich, S. 1991. A fast method for high-quality genomic DNA extraction from whole human blood. *BioTechniques* 11:298-301.
 39. Hance, A. J., B. Grandchamp, V. Lévy-Frébault, D. Lecossier, J. Rauzler, D. Bocart, and B. Gicquel. 1989. Detection and identification of mycobacteria by amplification of mycobacterial DNA. *Mol. Microbiol.* 3:843-849.
 40. Helmdahl, A., G. Hall, M. Hedberg, H. Sandberg, P. Söder, K. Tunér, and C. E. Nord. 1990. Detection and quantitation by lysis-filtration of bacteremia after different oral surgical procedures. *J. Clin. Microbiol.* 28:2205-2209.
 41. Hermans, P. W. M., R. A. Hartskeerl, J. E. Thole, and P. R. Klatser. 1990. Development of diagnostic tests for leprosy and tuberculosis. *Trop. Med. Parasitol.* 41:301-303.
 42. Higuchi, R. 1989. Simple and rapid preparation of samples for PCR, p. 31-38. In H. A. Erlich (ed.), *PCR Technology: Principles and Applications for DNA Amplification*. Stockton Press, New York.
 43. Hubbard, R. C., N. G. McElvaney, P. Birrer, S. Shak, W. W. Robinson, C. Jolley, M. Wu, M. S. Chernick, and R. G. Crystal. 1992. A preliminary study of aerosolized recombinant human deoxyribonuclease I in the treatment of cystic fibrosis. *N. Engl. J. Med.* 326:812-815.
 44. Hunsaker, W. R., H. Badri, M. Lombardo, and M. L. Collins. 1989. Nucleic acid hybridization assays employing dA-tailed capture probes. *Anal. Biochem.* 181:360-370.
 45. Ishizawa, M. Y., T. Kobayashi, and S. Matsuura. 1991. Simple procedure of DNA isolation from human serum. *Nucleic Acids Res.* 19:5792.
 46. Izraeli, S., C. Pfeiderer, and T. Lion. 1991. Detection of gene expression by PCR amplification of RNA derived from frozen heparinized whole blood. *Nucleic Acids Res.* 19:6051.
 47. Jiang, X., J. Wang, D. Y. Graham, and M. K. Estes. 1992. Detection of Norwalk virus in stool by polymerase chain reaction. *J. Clin. Microbiol.* 30:2529-2534.
 48. Jung, J. M., C. T. Comey, D. B. Baer, and B. Budowle. 1991. Extraction strategy for obtaining DNA from blood stains for PCR amplification and typing of the HLA-DQ α gene. *Int. J. Legal Med.* 10:145-148.
 49. Kain, K. C., A. E. Brown, H. K. Webster, R. A. Wirtz, J. S. Keystone, M. H. Rodriguez, J. Kinahan, M. Rowland, and D. E. Lanar. 1992. Circumsporozoite genotyping of global isolates of *Plasmodium vivax* from dried blood specimens. *J. Clin. Microbiol.* 30:1863-1866.
 50. Kaneko, K., O. Onodera, M. Miyatake, and S. Tsuji. 1990. Rapid diagnosis of tuberculosis meningitis by polymerase chain reaction. *Neurology* 40:1617-1618.
 51. Kwok, S., D. H. Mack, K. B. Mullis, B. Poiesz, G. Ehrlich, D. Blair, A. Friedman-Klen, and J. J. Sninsky. 1987. Identification of human immunodeficiency virus sequences by using in vitro enzymatic amplification and oligomer cleavage detection. *J. Virol.* 61:1690-1694.
 52. Lahiri, D. K., and J. I. Nurnberger, Jr. 1991. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res.* 19:5444.
 53. Lamblin, G., H. Rahmoune, J.-M. Wieruszkeski, M. Lhermitte, G. Sirecker, and P. Roussel. 1991. Structure of two sulphated oligosaccharides from respiratory mucins of a patient suffering from cystic fibrosis: a fast-atom-bombardment m.s. and ^1H -n.m.r. spectroscopic study. *Biochem. J.* 275:199-206.
 54. Lanciotti, R. S., C. H. Calisher, D. J. Gubler, G.-J. Chang, and A. V. Vornadam. 1992.

- Rapid detection and typing of Dengue viruses from clinical samples by using reverse transcription-polymerase chain reaction. *J. Clin. Microbiol.* 30:545-551.
55. Lebech, A.-M., and K. Hansen. 1992. Detection of *Borrelia burgdorferi* DNA in urine samples and cerebrospinal fluid samples from patients with early and late Lyme neuroborreliosis by polymerase chain reaction. *J. Clin. Microbiol.* 30:1646-1653.
 56. Lin, L., Y. Gong, G. D. Cimino, J. E. Hearst, and S. T. Isaacs. 1990. Two novel, rapid, high yield sample preparation methods for the PCR. *Abstr. 5th Conf. Nucleic Acids.*
 57. Loeffelholz, M. J., C. A. Lewinski, S. R. Silver, A. P. Purohit, S. A. Herman, D. A. Buonagurio, and E. A. Dragon. 1992. Detection of *Chlamydia trachomatis* in endocervical specimens by polymerase chain reaction. *J. Clin. Microbiol.* 30:2847-2851.
 58. Lund, A., Y. Wasteson, and Ø. Olsvik. 1991. Immunomagnetic separation and DNA hybridization for detection of enterotoxigenic *Escherichia coli* in a piglet model. *J. Clin. Microbiol.* 29:2259-2262.
 59. Marko, M. A., R. Chipperfield, and H. C. Birnboim. 1982. A procedure for the large-scale isolation of highly purified plasmid DNA using alkaline extraction and binding to glass powder. *Anal. Biochem.* 121:382-387.
 60. McCaustland, K. A., S. Bl, M. A. Purdy, and D. W. Bradley. 1991. Application of two RNA extraction methods prior to amplification of hepatitis E virus nucleic acid by the polymerase chain reaction. *J. Virol. Methods* 35:331-342.
 61. McHale, R. H., P. M. Stapleton, and P. L. Bergquist. 1991. Rapid preparation of blood and tissue samples for polymerase chain reaction. *BioTechniques* 10:20-22.
 62. McKenzie, H. A., and F. H. White, Jr. 1991. Lysozyme and α -lactalbumin: structure, function, and interrelationships. *Adv. Protein Chem.* 41:173-315.
 63. Mullis, K. B. 1991. The polymerase chain reaction in an anemic mode: how to avoid oligodeoxynucleotide fusion. *PCR Methods Applic.* 1:1-4.
 64. Noordhoek, G. T., E. C. Wolters, M. E. J. de Jonge, and J. D. A. van Embden. 1991. Detection by polymerase chain reaction of *Treponema pallidum* DNA in cerebrospinal fluid from neurosyphilis patients before and after antibiotic treatment. *J. Clin. Microbiol.* 29:1976-1984.
 65. Panaccio, M., and A. Lew. 1991. PCR based diagnosis in the presence of 8% (v/v) blood. *Nucleic Acids Res.* 19:1151.
 66. Persing, D. H., D. Mathiesen, W. F. Marshall, S. R. Telford, A. Spielman, J. W. Thomford, and P. A. Conrad. 1992. Detection of *Babesia microti* by polymerase chain reaction. *J. Clin. Microbiol.* 30:2097-2103.
 67. Pierre, C., D. Lecossier, Y. Bousougant, D. Bocart, V. Joly, P. Yenl, and A. J. Hance. 1991. Use of a reamplification protocol improves sensitivity of detection of *Mycobacterium tuberculosis* in clinical samples by amplification of DNA. *J. Clin. Microbiol.* 29:712-717.
 68. Ravaggi, A., D. Primi, and E. Cariani. 1992. Direct PCR amplification of HCV RNA from human serum. *PCR Methods Applic.* 1:291-292.
 69. Reddy, G. R., and J. B. Dame. 1992. rRNA-based method for sensitive detection of *Babesia bigemina* in bovine blood. *J. Clin. Microbiol.* 30:1811-1814.
 70. Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239:487-491.
 71. Saulnier, P., and A. Andremon. 1992. Detection of genes in feces by booster polymerase chain reaction. *J. Clin. Microbiol.* 30:2080-2083.
 72. Shak, S., D. J. Capon, R. Hellmuth, S. A. Marsters, and C. L. Baker. 1990. Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum. *Proc. Natl. Acad. Sci. USA* 87:9188-9192.
 73. Shankar, P., N. Manjunath, K. K. Mohan, K. Prasad, M. Behari, Shrinivas, and G. K. Ahuja. 1991. Rapid diagnosis of tuberculous meningitis by polymerase chain reaction. *Lancet* 337:5-7.
 74. Shirai, H., M. Nishibuchi, T. Ramamurthy, S. K. Bhattacharya, S. C. Pal, and Y.

0022-3000/97/080138-00